Analysis of Hypnotic Drugs by HPLC

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Pharmaceutical

Barbiturates are derivatives of barbituric acid in which alkyl-, aryl- or cycloalkyl groups substitute the H-atoms at C_5. The hypnotic effect increases with the number of C-atoms. Taken in medical doses the barbiturates cause a deep sleep but do not affect respiration, circulation and metabolism. Taken in higher doses, especially intravenous, they have narcotic effects which can lead to unconsciousness and cardiac arrest.

Figure 1 shows the separation of the eight hypnotic drugs barbital, allobarbital, phenobarbital, butabarbital, butalbital, amobarbital, mephobarbital and flunitrazepam using gradient analysis on a reversed phase column and UV detection.

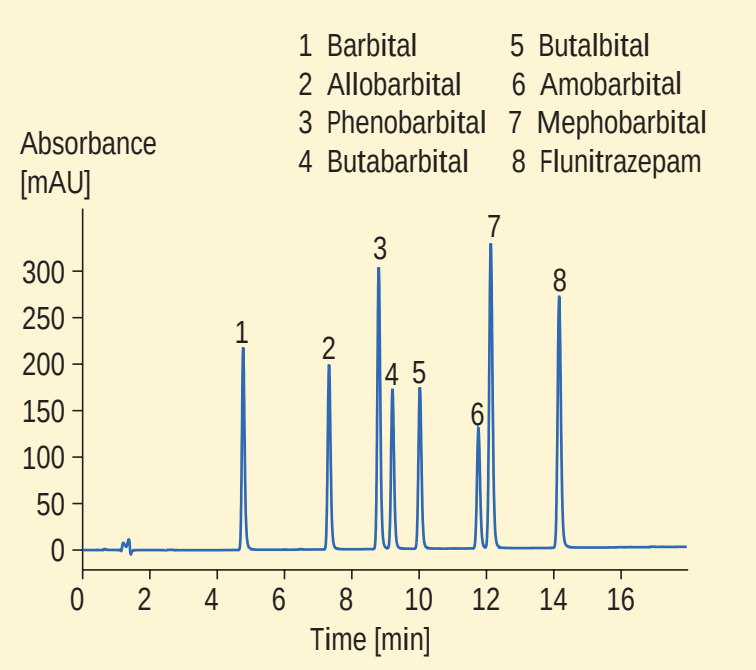

Figure 1
Analysis of eight hypnotic drugs

Conditions

Column
4 x 125 mm Hypersil ODS, 5 µm
Mobile phase
A = water, B = acetonitrile
Flow rate
1.0 ml/min
Gradient
at 0 min 10 % B
at 16 min 50 % B
Column wash
at 18 min 10 % B
UV detector
variable wavelength detector
204 nm, standard cell
Column compartment temperature
25 °C
Stop time
18 min
Post time
5 min
Injection volume
5 µl

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HPLC Performance

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs) (100 mg/l)*	Precision of Area (RSD of 10 runs) (100 mg/l)*
Barbital	0.2	0.05	0.50
Allobarbital	0.2	0.05	0.38
Phenobarbital	0.2	0.05	0.36
Butabarbital	0.2	0.03	0.25
Butalbital	0.4	0.03	0.25
Amobarbital	0.4	0.02	0.25
Mephobarbital	0.2	0.02	0.25
Flunitrazepam	0.2	0.03	0.23

* Injection volume: 5 µl

The performance of the HPLC method is shown in the table above.

The HPLC method presented here shows an easy but reliable and precise analysis of the hypnotic drugs barbital, allobarbital, phenobarbital, butabarbital, butalbital, amobarbital, mephobarbital and flunitrazepam. The values for LOD, precision of RT and precision of area show the good performance of the analysis.

Equipment

Agilent 1100 Series
- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume

Alternative:
- Binary pump
- Vacuum degasser
- Diode array detector standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns
- Hypersil ODS, 5 µm, 4 x 125 mm (Agilent part number 7992618-564)
- Recommended: Guard cartridges Hypersil ODS, 5 µm, 4 x 4 mm (Agilent part number 7992618-504, 10/pk)

Note:
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance.

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